A Broad-spectrum *Chromobacterium* Biolarvicide Formulation Disrupts Mosquito Development Through Gut Damage and Apoptotic Cell Death and Compromises Vector Competence

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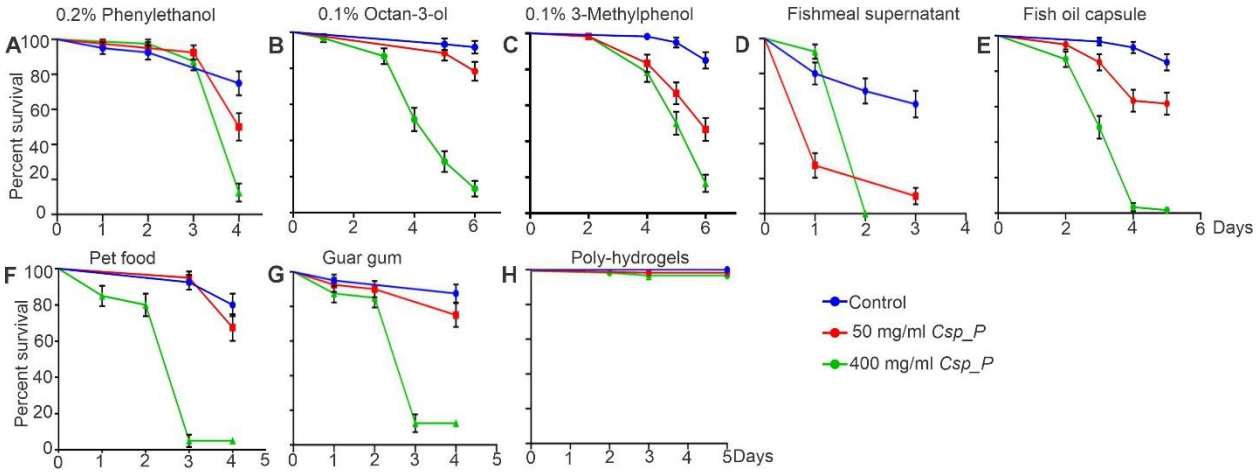
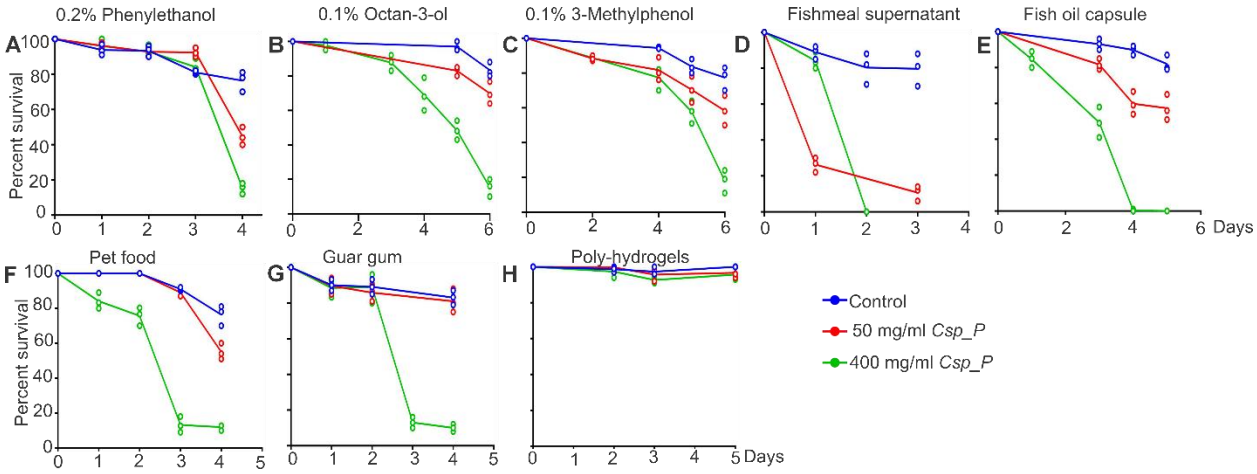
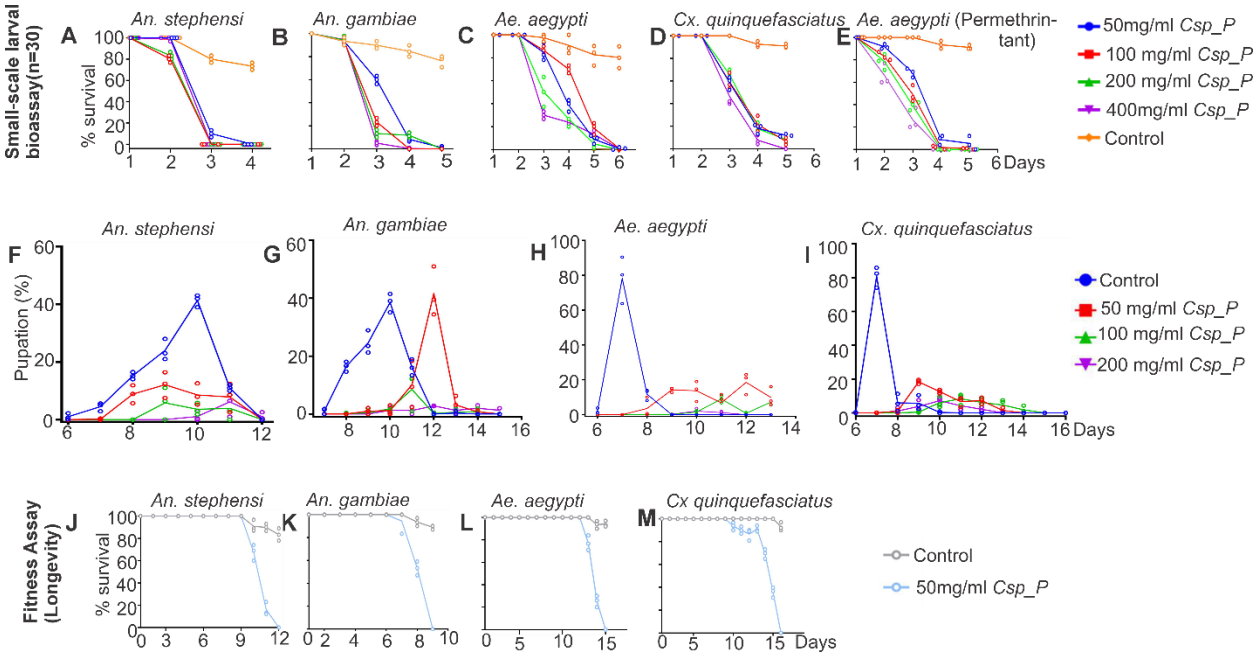


Supplementary Figure 1. Attractive larval baits used in the making of water-stable *Csp_P* pellets and assessment of their effect on the survival of *Ae. aegypti* larvae. (A-C) Chemical larval attractants, namely phenylethanol, octan-3-ol, and 3-methylphenol, were used in 50 and 400 mg/ml *Csp_P* pellets. The 400 mg/ml dosage of these pellets killed larvae in 4 to 6 days**. (D-F)** Pellets containing biological attractants killed larvae effectively at both (50 and 400 mg/mL). **(G-H)** Pellets with no attractant and formulations containing bio-hydrogels showed less or no mortality among the larvae exposed to them.



Supplementary Figure 1a. Attractive larval baits used in the making of water-stable *Csp_P* pellets and assessment of their effect on the survival of *Ae. aegypti* larvae. Survival (%) was monitored over time under control (blue), 50 mg/ml (red), and 400 mg/ml (green) *C.sp_P* treatments. Points represent individual biological replicates and lines indicate mean survival. Panels A–H correspond

to different attractants as labeled. For all graphs, circle points represent values for individual biological replicates and the lines at each assayed time point is the mean value of the biological replicates.



Supplementary Figure 2. *Csp_P* impacts mosquito survival, pupation and fitness (longevity). (A–E) Larval survival across species under different *Csp_P* concentrations. (F–I) % Pupation for *An. stephensi* (F), *An. gambiae* (G), *Ae. aegypti* (H), and *Cx. quinquefasciatus* (I). Data represent mean ± variation across independent biological replicates, with individual replicate values overlaid as points. (J–M) Survival of adult mosquitoes emerging from larvae exposed to *Csp_P* is shown for *An. stephensi* (J), *An. gambiae* (K), *Ae. aegypti* (L), and *Cx. quinquefasciatus* (M). Control (gray) and treated (blue, 50 mg/ml) groups are plotted over time. Curves represent mean survival across independent biological replicates. For all graphs, circle points represent values for individual biological replicates and the lines at each assayed time point is the mean value of the biological replicates.

Supplementary Table 1: Stability of various natural waxes and gums in the aqueous environment at various temperatures when combined with non-live *Csp_P* powder/fish meal as a pellet formulation.

S. No	Binder (%)	Day 7		Day 14		1 Month		2 Month	
		28°C	40°C	28°C	40°C	28°C	40°C	28°C	40°C
1	20% Candelilla wax	x	x	x	x	x	x	x	x
2	40% Candelilla wax	x	x	x	x	x	x	x	x
3	60% Candelilla wax	✓	✓	✓	✓	✓	✓	✓	x

4	20% Carnauba wax	✓	✓	✓	x	x	x	x	x
5	40% Carnauba wax	✓	✓	✓	x	x	x	x	x
6	20% Soy wax	x	x	x	x	x	x	x	x
7	60% Soy wax	✓	x	x	x	x	x	x	x
8	20% Paraffin wax	✓	✓	✓	x	✓	x	x	x
9	40% Paraffin wax	✓	✓	✓	✓	✓	✓	✓	x
10	40% Beeswax	x	x	x	x	x	x	x	x
11	40% Xanthan gum	x	x	x	x	x	x	x	x
12	40% Gum arabic	x	x	x	x	x	x	x	x
13	40% Edible gum	x	x	x	x	x	x	x	x
14	20% Guar gum	✓	✓	✓	✓	✓	✓	✓	✓
15	40% Guar gum	✓	✓	✓	✓	✓	✓	✓	✓

✓: stable; x: unstable

Supplementary Table 2. Primers used for apoptosis/ autophagic gene expression analysis using qPCR.

S.NO	Name	Gene ID	Primers sequence
1	*Ae*dronc F	AAEL011562	AGCTGGGATTTACCGTGTTC
2	*Ae*dronc R	AAEL011562	CGAAACATTCCGTCGATAGG
3	CASPS7 F	AAEL012143	CCAACATGGCAAGAAGTACG
4	CASPS7 R	AAEL012143	TTCTGCTGGTGCATCATAGG
5	CASPS8 F	AAEL014348	AAACGACCTCGATTCTGGTG
6	CASPS8 R	AAEL014348	AACCGTTCTTGGTGTTACGG
7	*Ae*IAP1 F	AAEL009074	AAAGTGATCGGGTCAAGTGC
8	*Ae*IAP1 R	AAEL009074	CGCCCTTCATTAGTTTCAGG
9	Mx F	AAEL014196	AGCAAACGTTGTTCGAGCTG
10	Mx R	AAEL014196	ACAGGGCGTTGTCTGGATTC
11	Imp F	AAEL004392	ACTGTGCCCTACATGGAAGC
12	Imp R	AAEL004392	CGTGTACCGGTAGAGCACCT
13	IAP5 F	AAEL014251	GGTTTCTACTGGCAGGGTGA
14	IAP5 R	AAEL014251	AGCGTGCTTCTTGTGTTCCT
15	IAP9 F	AAEL012512	CGACAAAATGGGTAGGGAAA
16	IAP9 R	AAEL012512	GCCTTCACCGGTGTTATCAT
17	*Ae*DNR1 F	AAEL000590	CATGAACGAAGAGCATGAGG

18	*Ae*DNR1 R	AAEL000590	GTAATGGCCCGGTAGAGACA
19	*Ae*BRUCE F	AAEL012446	CTGAAGCAGCACGAGAATCA
20	*Ae*BRUCE R	AAEL012446	ACTGGGAACTCCTCGAACCT
21	ATG1 F	AAEL016987	CACTCAGCGAGGACACGATA
22	ATG1 R	AAEL016987	GTCGGCTATTTTGAGGGTGA
23	ATG4A F	AAEL010516	CCGAAGCATTCGCTGTACTT
24	ATG4A R	AAEL010516	CGGAAAGTTCTCCTGGTTGA
25	ATG5 F	AAEL002286	GGATGGACAGCAATGGAACT
26	ATG5 R	AAEL002286	AAGTGGGCCTCAACAATGTC
27	ATG8 F	AAEL007162	GTGAGCAAGCGTCAAATCTC
28	ATG8 R	AAEL007162	GGATACAGTCCGCCGATAAA
29	ATG12 F	AAEL009089	ATTCTCCATGCAACGGGTAG
30	ATG12 R	AAEL009089	TATCTGATCTGGCGATGGTG
31	ATG7 F	AAEL009814	TGGGGAGCTCAAAACGTATC
32	ATG7 R	AAEL009814	GCCTTAGGCTTTCCTCCATT
33	*Ae*p53 F	AAEL007595	CCGTCATGGAGGAGTCATCT
34	*Ae*p53 R	AAEL007595	GCGGAAGTTTGTGAGTGTGA
35	*Ae*decbl F	AAEL001515	GATGACATCGCCAACACTTG
36	*Ae*decbl R	AAEL001515	TCCCCACAGAGACATTTTCC
37	*Ae*buffy F	AAEL001521	AAGTCCGGCGTGTTCAATAG
38	*Ae*buffy R	AAEL001521	CCGTTGTAAACCCGAGGATA
39	RPL8 F	AAEL000987	TGGGGCGTGTCATTCGTGCACAG
40	RPL8 R	AAEL000987	GGTATCCGTGACGTTCGGCA
41	RPS17 F	AAEL004175	AGACAACTACGTGCCGGAAG
42	RPS17 R	AAEL004175	TTGGTGACCTGGACAACGATG

Supplementary Table 3. Egg-laying success and fecundity following larval *Csp_P* exposure.

Treatment	Females (n)	Females laying egg (%)	Eggs per laying female (mean ± SEM)
Control (*An. gambiae*)	30	23 (77%)	38±19.6
Csp_P (*An. gambiae*)	25	9 (36%)	11.3±12.7
Control (*Ae. aegypti*)	52	44 (85%)	67.0±40.0
Csp_P (*Ae. aegypti*)	52	22 (42%)	42.1±29.4

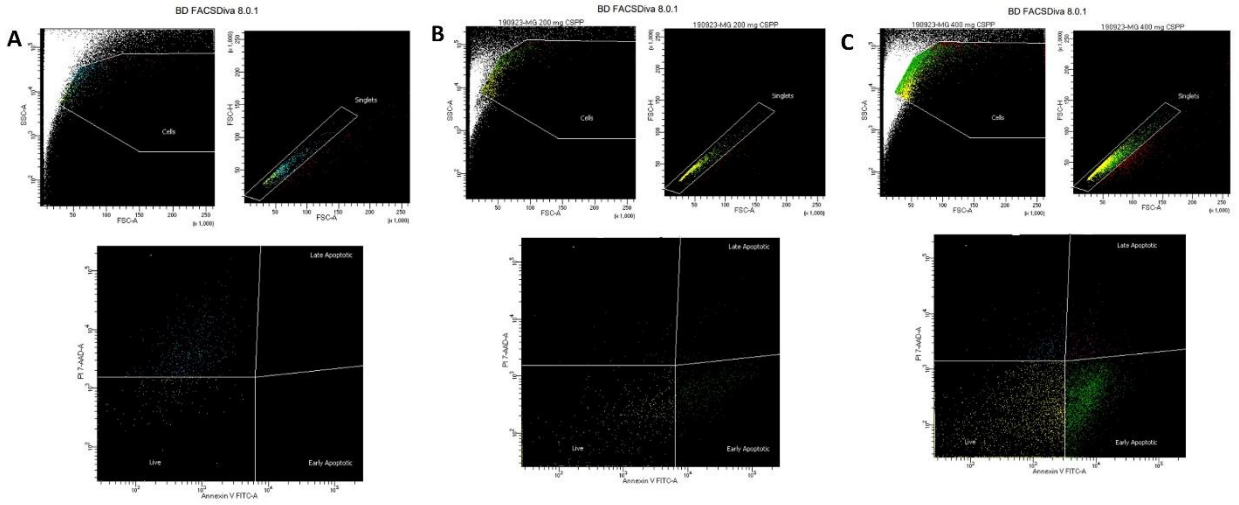
*Egg numbers are reported as mean ± SEM and were calculated only for females that laid eggs.

Supplementary Table 4. Infection prevalence and oocyst burden following larval *Csp_P* exposure.

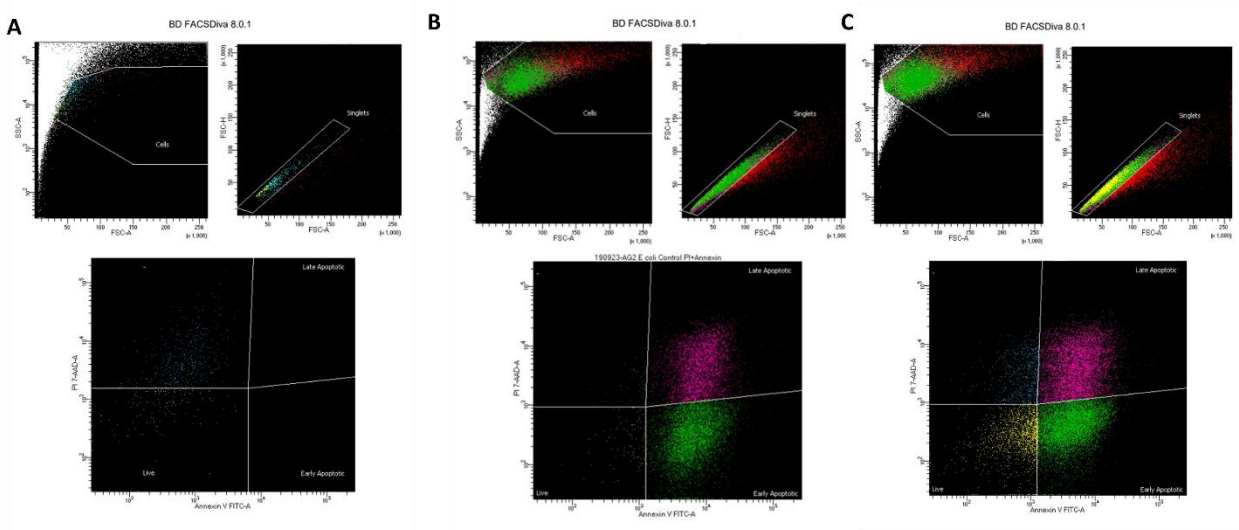
Treatment	Females (n)	Infected Females (%)	Oocysts/pfu per infected mosquito (mean ± SEM)
Control (*An. gambiae*)	31	29 (94%)	47.0±29.4
Csp_P (An. gambiae)	31	23 (74%)	13.4±22.8

Control (*Ae. aegypti*)	74	54 (73%)	2.9±0.4
Csp_P (Ae. aegypti)	60	28 (47%)	1.7±0.3

*Oocyst/pfu numbers are reported as mean ± SEM and were calculated only for females that are infected.



Supplementary Figure 3. Flow cytometric analysis of apoptosis by Annexin V/PI staining. (A) Representative flow cytometry plots of midgut-derived cells from control-treated larvae. Upper right panel I shows the total event population (including debris), with the gated region indicating intact cells after exclusion of debris. Upper left panel II shows singlet discrimination to eliminate doublets. Lower panel III displays Annexin V–FITC versus PI staining, with quadrants identifying live (Annexin V^-/PI^-), early apoptotic (Annexin V^+/PI^-), late apoptotic (Annexin V^+/PI^+), and necrotic (Annexin V^-/PI^+) cell populations. **(B–C)** Representative flow cytometry plots from Csp_P-treated larvae. The same sequential gating strategy was applied to quantify apoptotic and necrotic cell populations.



Supplementary Figure 4. Flow cytometric analysis of DNA fragmentation by TUNEL assay. (A) Representative flow cytometry plots showing DNA fragmentation in midgut-derived cells isolated from

control treated larvae; **(B-C)** flow cytometry plots from Csp_P-treated larvae. The upper panels depict sequential gating strategy: total events (including debris), gated cell population after debris exclusion, and singlet discrimination. The lower panels show fluorescence intensity corresponding to DNA fragmentation, with fragmented DNA-positive cells quantified within the gated singlet population**.**